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Influence of food additives on the control of postharvest rots of apple and peach and efficacy of the yeast-based biocontrol product Aspire

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Abstract

The effects of calcium propionate, sodium bicarbonate, and sodium ethylenediamine-tetraacetic acid (EDTA) on postharvest pathogens of apple and peach, and on improving the efficacy of the biocontrol product Aspire were evaluated. All three materials had a distinct inhibitory effect on the radial growth of *Botrytis cinerea* and *Penicillium expansum* in vitro. The inhibitory effect increased with the concentration of each material. When tested on apple, sodium bicarbonate and EDTA exhibited only a curative effect (control of pre-existing infections) against infections caused by *B. cinerea*. Ca-propionate, however, provided both protective and curative effects against infections caused by *B. cinerea*. In the case of *P. expansum,* sodium bicarbonate, at concentrations up to 0.4% and EDTA, at all concentrations tested, failed to provide any curative or protective activity against infections by *P. expansum* on apple. Sodium bicarbonate at 2% was the most consistent in providing a measure of fungicidal activity when used alone. In combination with Aspire, 2% sodium bicarbonate exhibited a consistent ability to significantly enhance its biocontrol performance (curative and protective effect) against *Botrytis* and *Penicillium* rot in apple and *Monilinia* and *Rhizopus* rot in peach. Based on these results, it appears that the use of additives, such as sodium bicarbonate, is a useful approach to improve the efficacy of yeast antagonists used for postharvest disease control.

Keywords: *Prunus persica*; *Malus domestica*; *Candidla oleophila*; Biological control; Sodium bicarbonate; Calcium propionate; EDTA

1. Introduction

Considerable research effort has been devoted to identifying yeast organisms that effectively control postharvest diseases of fruit, vegetables, and grains (Wilson et al., 1996). At least two yeast-based products are now commercially available

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Aspire (Ecogen, Inc., Langhorne, PA) containing *Candida oleophila*, and Yield Plus (Anchor Yeast, Cape Town, South Africa) containing *Cryptococcus albidus*. Several more are in the advanced stages of commercialization (El Ghaouth et al., 2000a,b). The products Biosave-110 and Biosave-111 (EcoScience, New Brunswick, NJ), containing the bacterium, *Pseudomonas syringae*, are also available for postharvest disease control. Although antagonistic yeasts have been shown to protect a variety of fruit, their efficacy under semi-commercial conditions is sometimes lower than chemical control (Droby et al., 1993, 1998; El Ghaouth et al., 2000a,b). In large-scale tests, the use of antagonists often needs to be supplemented with low doses of synthetic fungicides in order to provide a level of disease control equivalent to synthetic fungicides (Wilson and Wisniewski, 1994; Brown and Chambers, 1996; Droby et al., 1998). Factors involved in decreasing efficacy and inconsistency of biocontrol agents under commercial conditions have not yet been fully identified. It is believed, however, that the inability of yeast antagonists to effectively control pre-existing infections inflicted during harvesting and transport is the major reason for a lack of adequate control.

A principal goal in the development and implementation of successful biological control products is improving the ability of yeast antagonists to successfully control postharvest diseases under a wider array of conditions and with minimal variability. Additionally, the ability to control pre-existing infections, as is possible with synthetic fungicides, is also highly desired (El Ghaouth et al., 2000a,b). Enhancing the activity of biocontrol agents could be the most important factor in their success in controlling fruit diseases and their ultimate acceptance in commercial disease management.

McLaughlin et al. (1990) demonstrated that the addition of calcium salts to yeast cell suspensions markedly enhanced the ability of *P. guilliermondii* to control postharvest diseases of apple. This allowed a reduction in the amount of yeast biomass needed to achieve desirable levels of disease control. Wisniewski et al. (1995) reported that biocontrol activity of isolate 182 of the yeast *C. oleophila* was also enhanced by the addition of 90 or 180 mM $CaCl_2$. The combination of sugar analogs like 2-deoxy-D-glucose with the yeast antagonists *Sporobolomyces roseus* or *C. saitoana* enhanced biocontrol against blue mold of apples (Janisiewicz, 1994; El Ghaouth et al., 2000a).

The present study aimed at evaluating the effect of various preservative materials, widely used in the food industry, on postharvest pathogens of apple and peach, and exploring the potential of using them to enhance the efficacy of the biocontrol product Aspire. This was done by identifying the compounds that exhibited the ability to control pre-existing infections (curative activity), as well as to enhance the protective activity of the biocontrol agent.

2. Materials and methods

2.1. Plant material, fungal cultures and chemicals

'Golden Delicious' apples and 'Loring' peaches were obtained from the USDA-ARS, Appalachian Fruit Research Station, Kearneysville, WV, USA during the summer and fall of 1999 and either used immediately or stored at 4 °C until use. Before treatments, fruit were washed in tap water and allowed to dry over night. Experiments were repeated at least three times with 80 wounds (20 apples, four wounds per fruit) per treatment in each experiment. *Penicillium expansum* and *Botrytis cinerea* were obtained from infected fruits and cultured on potato dextrose agar (Difco, Detroit, MI). Spore suspensions were prepared by flooding a Petri plate containing a 2–3-week-old sporulating culture with sterile distilled water. The spore concentration was determined with a haemocytometer and adjusted as required. The product Aspire (Ecogen, Langhorne, PA), based on the yeast *C. oleophila* (strain 182), was used according to label instructions. Aspire was used at a rate of 2.4 mg ml^{-1} distilled water or additive solution. Aspire was re-hydrated by stirring the mixture for 20 min at room temperature on a magnetic stirrer. This yielded a concentration of 1×10^8 colony forming units (cfu) ml^{-1}. Twenty-five μl of the prepared Aspire were added to each wound.

Sodium bicarbonate ($NaHCO_3$, Sigma, St. Louis, MO), calcium propionate ($C_6H_{10}CaO_4$, Sigma) and sodium ethylenediamine-tetraacetic acid, di-sodium salt (Na-ETDA, $C_{10}H_{14}N_2O_8Na_2 \cdot 2H_2O$, Sigma) were the additives that were used alone or in combination with Aspire.

2.2. In vitro inhibition of growth of B. cinerea and P. expansum

To assess the effect of the various additives on the growth of the pathogens, agar plugs (5 mm in diameter) containing mycelia from the growing edge of 1-week-old cultures of *P. expansum* or *B. cinerea* were placed in the center of petri dishes containing Difco (Detroit, MI) yeast maltose agar (YMA) amended with various test salts at different concentrations. Salt solutions were filtered through a 0.45 μm millipore filter before adding them to autoclaved YMA after it had cooled to 55 °C. Treatments were set up in triplicate and radial growth was monitored at 2 and 6 days. Results are reported as the percent inhibition relative to growth in control (YMA) petri plates not containing the additives.

2.3. Tests on fruit

After being arranged on packing trays in plastic tubs, the fruit were wounded with four wounds to each fruit. Wounds were approximately 3 mm in diameter and 3–4 mm deep. The wounds were allowed to dry for 2 h and then 25 μl of each treatment was pipetted into each wound. Wounds treated with water served as controls. For protectant activity tests the treatments were applied to the wounds 2 h prior to the application of the pathogen, which was applied as a 25 μl suspension of spores in aqueous Tween-20 (0.05%). For curative activity tests the pathogen was applied 24 h prior to the treatment solutions. Pathogens used in the apple studies were *B. cinerea* and *P. expansum*. Treated fruit were stored in covered plastic trays at ambient temperatures (20–22 °C) with 100 ml of water added to the tub to maintain high humidity. The percentage of infected wounds was determined at 7–21 days after inoculation. Each treatment consisted of 80 wounds (20 apples with four wounds per fruit).

For the peach tests, the effect of treatment solutions was assessed against the development of natural infection of surface wounds in 'Loring' peach by *Monilinia fructicola* and *Rhizopus stolonifer*. After wounding, fruit were dipped for 20 s in the appropriate treatment solution (additives alone or combined with the yeast cells). Wounded fruit dipped in water were used as controls. After allowing the fruit to air-dry for 2–3 h, the fruit were placed in covered plastic trays and the percentage of infected wounds in treated fruit was compared with control fruit after 3–5 days of incubation at room temperature. Each treatment contained three replicates of 10 fruit each.

2.4. Effect of food additives on growth of C. oleophila in surface wounds

Growth of *C. oleophila* was followed in apple wounds inoculated with 20 μl of fresh (shake culture) yeast cell suspension at a concentration of 1×10^8 cfu ml^{-1} with or without the additives. Inoculated wounds were sampled with a cork borer at 0, 6, 24, and 120 h and homogenized in 5 ml of sterile water. After serial dilutions, aliquots of 100 μl were plated on YMA petri plates and the numbers of the colonies counted after 48 h of incubation at 25 °C. Each fruit, containing three wounds, represented a single replicate, and each treatment was replicated three times. Data are presented as cfu ml^{-1}. The experiment was repeated three times.

3. Results and discussion

Based on prior preliminary screening tests (Wisniewski et al., 1998), we examined the effect of calcium propionate (CaP), sodium bicarbonate (NaBi), and Na-EDTA on apple and peach pathogens to see if they enhanced the efficacy of the commercial product, Aspire (containing *C. oleophila* strain 182). All three additives inhibited radial growth of *B. cinerea* and *P. expansum* in vitro (Fig. 1). In all cases, the inhibitory effect on growth increased with concentration of the addi-

tive. The inhibitory effect of NaBi was evident at relatively low concentrations (0.3–0.6%, w/v) compared with the concentration of CaP (2–5%, w/v). Na-EDTA significantly inhibited growth of both pathogens at concentrations of 5 mM (0.19% w/v)) and higher. When tested on apples, NaBi and EDTA exhibited only curative effects against *B. cinerea*. Marked reduction in decay incidence was evident at concentrations of 0.2% and 5 mM of NaBi and EDTA, respectively (Fig. 2A–C). CaP,

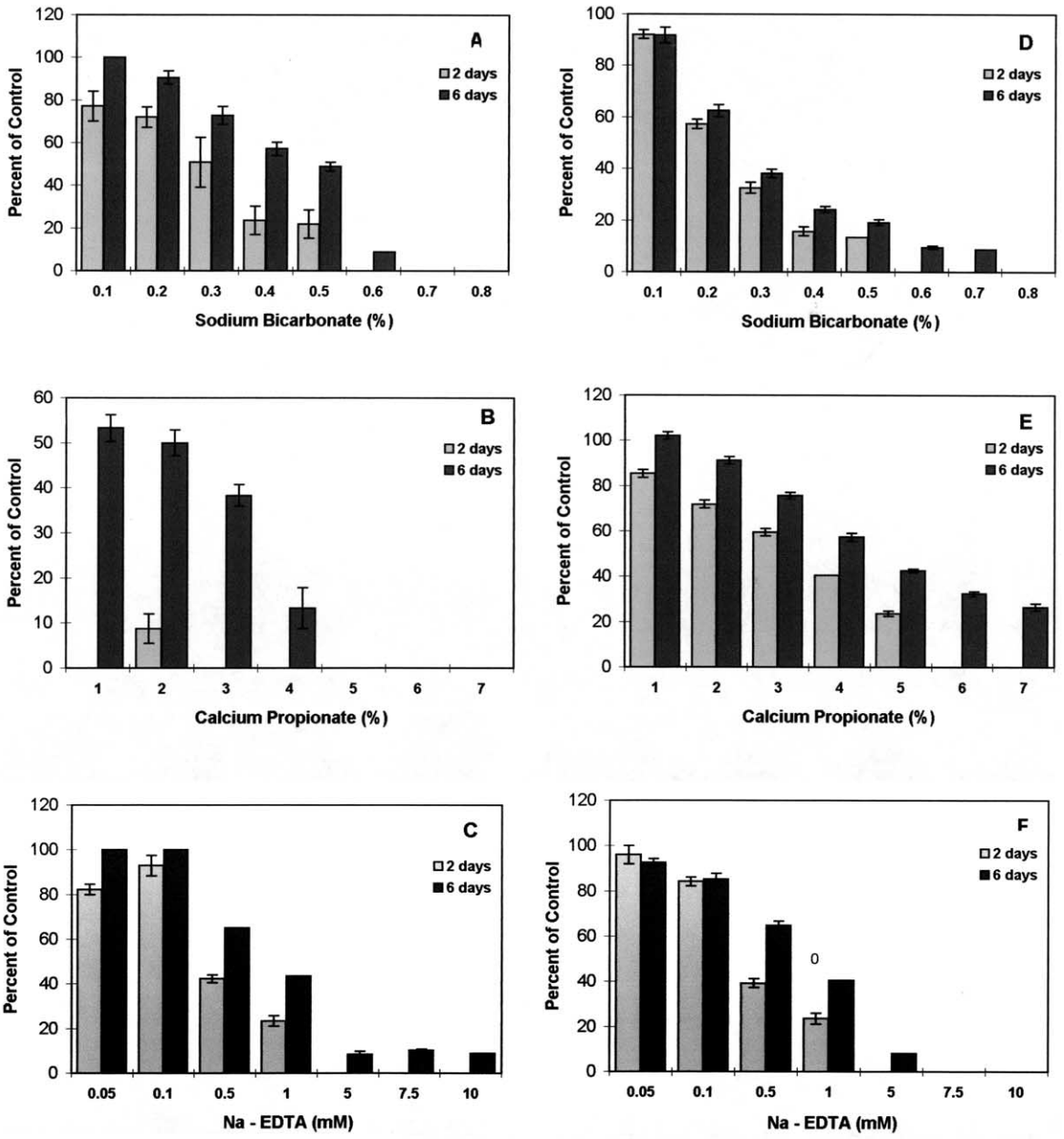

Fig. 1. Effect of various additives on radial growth (expressed as percent of control) of *Botrytis cinerea* (A–C) and *P. expansum* (D–F). $n = 9 \pm$S.E. Controls were cultures grown on yeast maltose agar without the addition of additives. Percent additives is w/v.

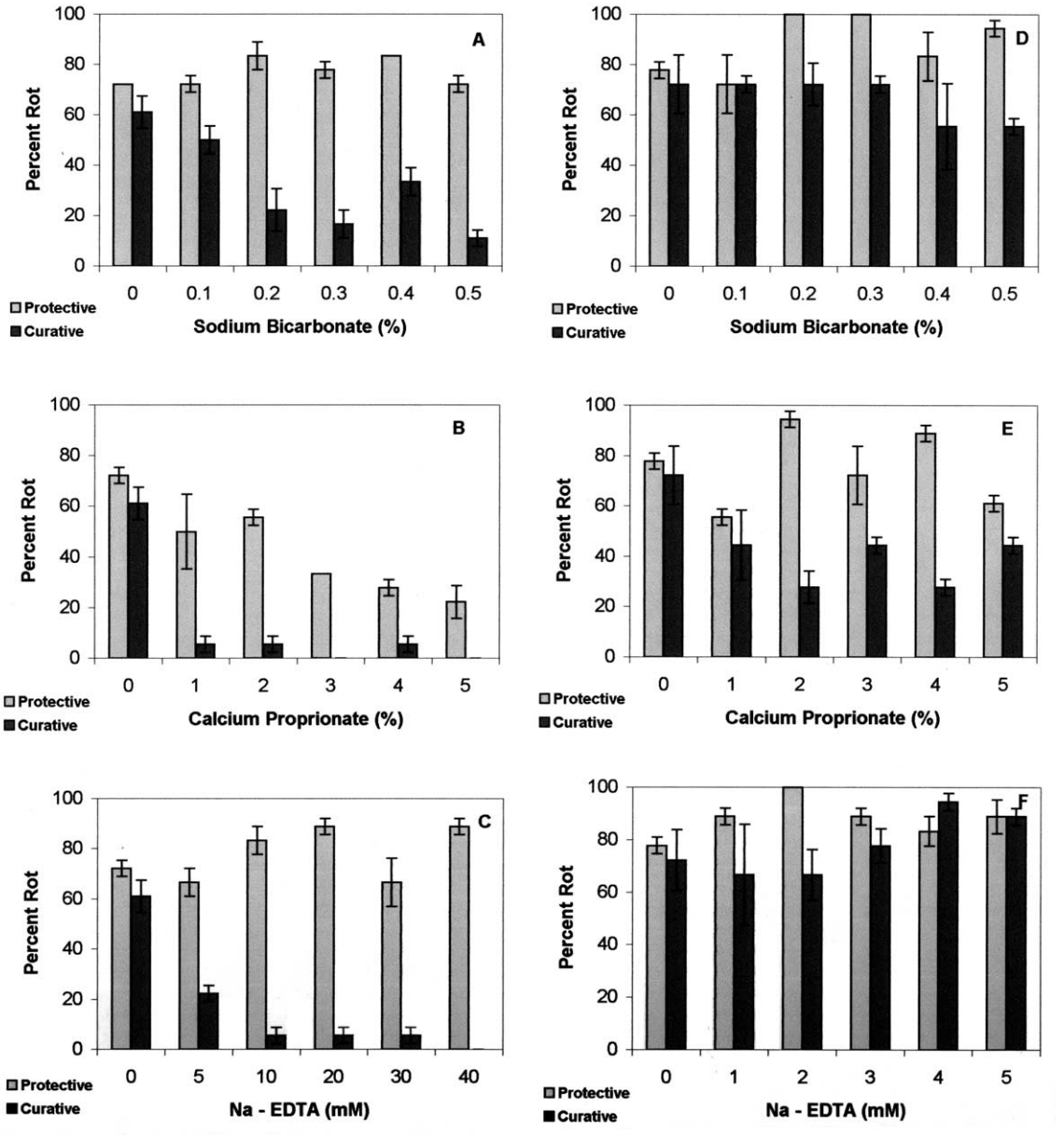

Fig. 2. Comparison of protective and curative activity of various additives against *Botrytis cinerea* (A–C) and *P. expansum* (D–F) in wounded apple fruit. $n = 80 \pm$S.E. Percent additives is w/v.

however, provided both protective and curative effects against *B. cinerea* (Fig. 2B). In the case of *P. expansum*, inhibition by NaBi, CaP and EDTA was less than that of *B. cinerea* (Fig. 2A–C). NaBi, at concentrations up to 0.4% and EDTA, at all concentrations tested, failed to provide any curative or protective activity against *P. expansum* on apple. Slight, but significant, curative activity of NaBi against *P. expansum* occurred at 0.5%. In contrast, CaP, at concentrations of 1% and above exhibited moderate curative activity against *P. expansum.*

The concentrations of NaBi that were inhibitory in vitro were not as effective in planta against both pathogens. For this reason, a series of subsequent tests on apples showed that 2% NaBi had both protective and curative activity against *B. cinerea* and *P. expansum* (data not shown).

Experiments to examine the protective enhancement of combinations of the additives with Aspire, revealed that the inhibitory effects of the additives was variable and that the use of 2% NaBi gave the most consistent positive results (data not shown). Only 2% NaBi enhanced the performance of Aspire in controlling *Botrytis* rot in apple (Fig. 3A–C). Similar variability was observed in experiments using *P. expansum* (Fig. 3D–F). The ability of 2% NaBi to enhance both the protective and curative activity of Aspire against *Botrytis* and *Penicillium* rots is illustrated in Fig. 4A and B. In the tray tests conducted, Aspire exhibited such effective control of *Penicillium* that the enhance-

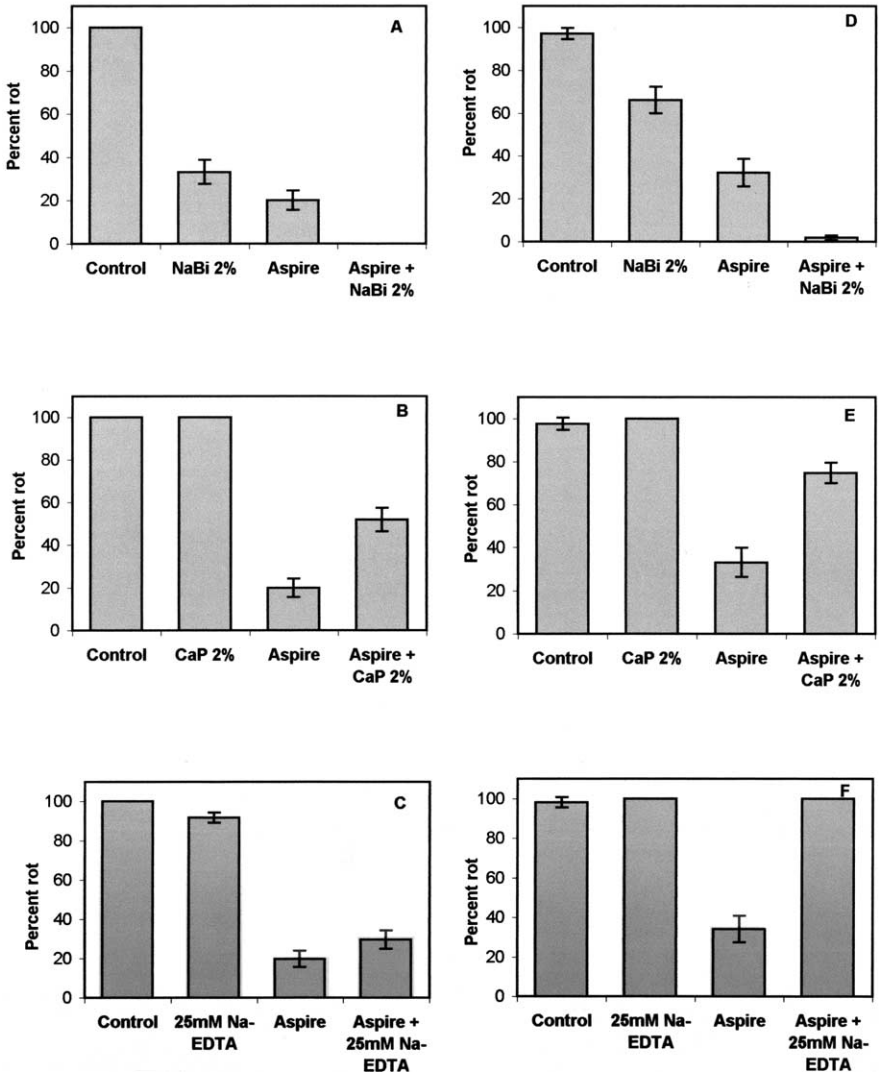

Fig. 3. Effect of various additives on the efficacy of Aspire against *Botrytis cinerea* (A–C) and *P. expansum* (D–F) on wounded apples. $n = 80 \pm$S.E. Percent additives is w/v. NaBi, sodium bicarbonate; CaP, calcium propionate.

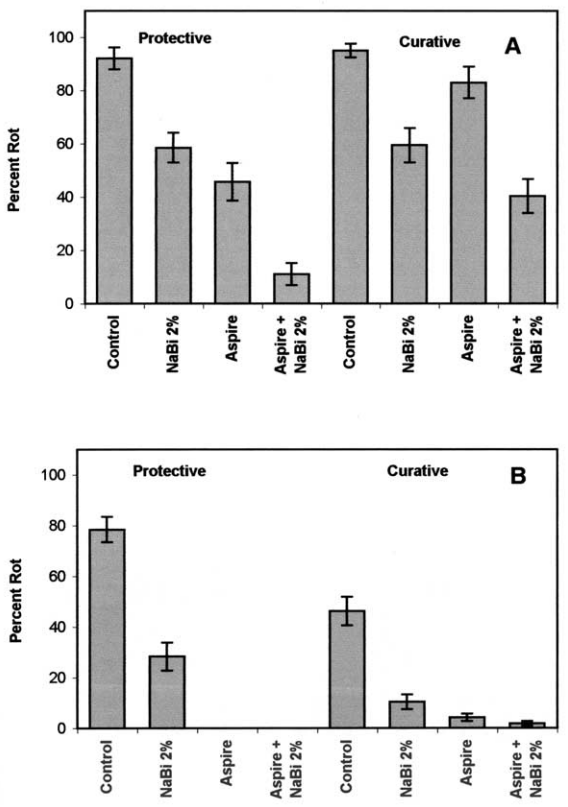

Fig. 4. Effect of various additives on the protective and curative activity of Aspire against *Botrytis cinerea* (A) and *P. expansum* (B) on wounded apples. $n = 20 \pm$S.E. Percent additives is w/v. NaBi, sodium bicarbonate; CaP, calcium propionate.

ment brought about by the use of the additives was only evident in the curative studies.

In peach, only the natural infection of wounds was observed and *Monilinia fructicola* and *Rhizopus stolonifer* predominated. Only 2% NaBi enhanced the biocontrol efficacy of Aspire (Fig. 5). Additionally, in contrast to apple, application of EDTA to peaches was phytotoxic and resulted in greatly enhanced decay of the fruit (data not shown).

The ability of *C. oleophila* (strain 182) to grow in wound sites in the presence of these additives was assessed (Fig. 6). The growth of the yeast was not significantly affected by any of the additives except EDTA at 20 mM, which had a slight inhibitory effect 120 h after treatment. It should be noted, however, that standing solutions (for 24 h) of the additives containing the yeast were much more inhibitory (data not shown), suggesting that in the wound site the additives are quickly absorbed or become bound to cell walls.

Smilanick et al. (1997) have demonstrated that treatment of citrus with warm (35–46 °C) solutions of sodium carbonate and bicarbonate salts is an effective method of reducing postharvest decay caused by green mold (*Penicillium digitatum*). The effectiveness of both salt solutions was significantly improved when these treatments were followed by the fungicide imazalil, or the biological control antagonist *P. syringae,* strain ESC10 (Smilanick and Soresenon, 2000). This work showed that the combination of sodium bicarbonate or sodium carbonate followed by the biological control antagonist *P. syringae* overcomes significant shortcomings of either of these treatments alone. Our study demonstrates that the use of sodium bicarbonate may also be applicable to the postharvest treatment of pome fruit. Additionally, combination of sodium bicarbonate salt and the biocontrol product, Aspire, which contains the yeast *C. oleophila* (strain 182) resulted in superior control compared with either treatment alone. The use of the carbonate salt, as opposed to the bicarbonate salt, was not compatible with Aspire

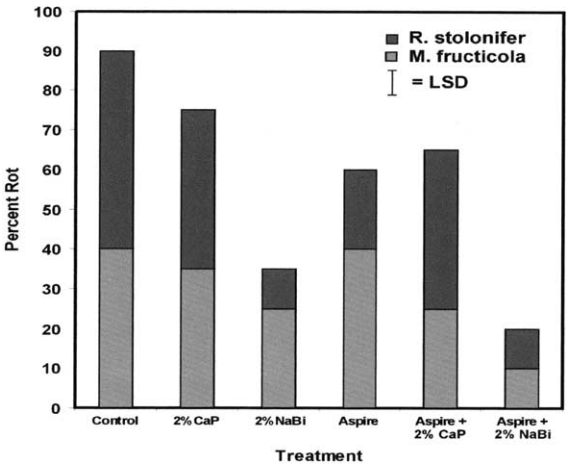

Fig. 5. Effect of various additives on the efficacy of Aspire against natural infections of peach fruit by *Monilinia fructicola* and *Rhizopus stolonifer*. $n = 30 \pm$S.E. Percent additives is w/v. NaBi, sodium bicarbonate; CaP, calcium propionate.

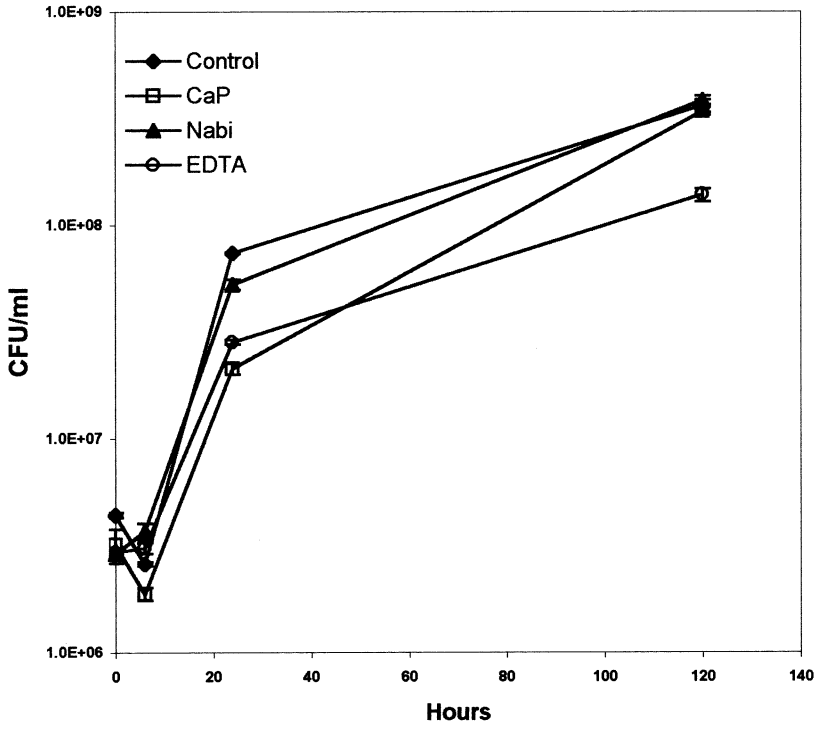

Fig. 6. Growth of the yeast, *C. oleophila* (as formulated in the product Aspire), in apple wounds when the product was applied alone or combined with either calcium propionate (CaP), sodium bicarbonate (NaBi), or EDTA. $n = 3 \pm$S.E. Percent additives is w/v.

due to the high pH of the carbonate solution, which was lethal to the yeast cells (data not shown).

Microbial antagonists have a poor ability to eradicate pre-existing infections, while chemical fungicides, sodium bicarbonate, and hot water treatment can control recently established (within 24 h) infections. Sodium bicarbonate and hot water treatments, however, do not exhibit persistent protection of the fruit from re-infection, while the application of microbial antagonists, applied together or after these treatments, protects surface wounds from re-infection. Clearly, further research is needed to identify the best method of utilizing these combinations (temperature, method of application, etc.). El Ghaouth et al. (2000a,b) have also reported enhancing the biocontrol efficacy of the yeast, *Candida saitoana*, by combining it with either glycochitosan, forming a 'bioactive coating', or with the sugar 2-deoxy-D-glucose. Both approaches increased the protective and curative activity of the yeast in controlling postharvest diseases. As efforts continue to find alternatives to use of synthetic fungicides to control postharvest losses, biological control will continue to be a viable approach, especially as it becomes integrated with other approaches such as the use of additives that enhance their performance over a wider range of conditions.

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